Spotted knapweed (Centaurea maculosa L.) seed longevity, chemical control and seed morphology
by Edward Stuart Davis

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
Spotted knapweed (Centaurea maculosa Lam.) is an introduced perennial plant that has spread rapidly to infest an estimated 4.7 million acres of grazeable rangeland, woodland and pastureland in Montana. Spotted knapweed is a primary invader that capitalizes on soil disturbances and once established is extremely competitive. Carrying capacity of rangeland is severely reduced as spotted knapweed displaces productive grasses and forbs.

Seed longevity is an important survival characteristic of spotted knapweed. Over 50% of a population of spotted knapweed seeds remained viable after 5 years of burial. In a natural seedbank, spotted knapweed seed populations declined by 95% during the first 3 years following termination of seed production. However, approximately 390,000 viable seeds remained per hectare 7 years after seed production had been terminated. In laboratory tests 10 to 40% of freshly harvested spotted knapweed seed required light to germinate.

Field trials were established to determine the long-term control of spotted knapweed achieved by a single application of low rates of picloram. All rates significantly reduced spotted knapweed densities 60 and 84 months after treatment at two locations. Grass forage production increased 200 to 700% when spotted knapweed was controlled. Spotted knapweed plants gradually reinfested the treated areas as picloram soil residues declined causing reduced grass production.

Spotted knapweed seeds rapidly imbibe water and germinate within 18 hours. Water uptake occurs primarily through the hilum and to a lesser degree through the micropyle of the seed. A thick, durable seed coat provides effective protection which permits dormant seed to resist decomposition in soil.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

9-12-90 — Date

Peter K Fay — Chairperson, Graduate Committee

Approved for the Major Department

9/12/90 — Date

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Approved for the College of Graduate Studies

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Date 17, Sept 1990

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ABSTRACT

Spotted knapweed (*Centaurea maculosa* Lam.) is an introduced perennial plant that has spread rapidly to infest an estimated 4.7 million acres of grazeable rangeland, woodland and pastureland in Montana. Spotted knapweed is a primary invader that capitalizes on soil disturbances and once established is extremely competitive. Carrying capacity of rangeland is severely reduced as spotted knapweed displaces productive grasses and forbs.

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CHAPTER 1

LITERATURE REVIEW

Introduction

Spotted knapweed (*Centaurea maculosa* Lam.) is a pernicious noxious weed in Montana infesting an estimated 4.7 million acres of range and grazeable woodland (Lacey, 1987). An additional 34 million acres may be vulnerable to spotted knapweed invasion (Chicoine, Fay, and Nielson, 1986). The annual forage loss due to displacement of native forbs and grasses by spotted knapweed is estimated at $4.5 million (French and Lacey, 1983).

The perennial growth habit, profuse seed production and aggressiveness of spotted knapweed result in rapid establishment and spread. Initial infestations occur in disturbed areas such as roadsides, trails, construction sites, overgrazed land and waterways (Watson and Renney, 1974). Once established it is very competitive, displacing native grasses and forbs, resulting in near-monoculture stands of spotted knapweed. Another factor contributing to its success in North America is the lack of natural enemies. In Europe, its center of origin, spotted knapweed evolved with host-specific insects and pathogens which keep the plant frequency and density at low levels. In North America most of these agents are not present.

Seed Germination and Seedling Establishment

Spotted and diffuse knapweed (*Centaurea diffusa* Lam.) seed is disseminated as a dry, indehiscent single seeded fruit called an achene. Members of the *Compositae* have epigynous flowers (flowers with an inferior ovary). The flower parts, calyx, corolla, and stamens originate above the inferior ovary. Achenes produced from epigynous flowers are termed cypselas. Throughout the text of this thesis, the term seed will be synonymous with achene or cypsela.

Knapweed seeds germinate in the fall and spring when soil moisture conditions are most favorable. Seedlings that germinate in the fall overwinter as rosettes and bolt the following June. Seedlings established in the spring usually flower the following season (Schirman, 1981).

Knapweed seeds imbibe water and germinate within 18 hours under optimum conditions (Chicoine, 1984). Spears et al. (1980) and Eddleman and Romo (1986) examined canopy cover, depth of emergence of seedlings, temperature and moisture and determined the optimum conditions for germination. Canopy cover had no effect on germination of buried seed. Maximum germination occured at soil moisture levels of 118 to 127% of field capacity in the soil mixture used at soil temperatures ranging from 10 to 28° C.

Seedlings did not emerge from depths of 3 and 5 cm for diffuse and spotted knapweed, respectively. The highest percent germination occurred when seeds were left on the soil surface.

Watson and Renney (1974) reported germination levels of freshly harvested diffuse and spotted knapweed seeds increased from 40 to 68% and from 20 to 80%, respectively, following 25 days of dry storage at room temperature. Their findings suggest that knapweed seeds possess a primary (innate) dormancy which is lost during dry after-ripening.

Nolan and Upadhyaya (1988) describe three types of germination behavior in freshly harvested diffuse and spotted knapweed seed: 1) nondormant seeds that germinate in darkness, 2) light-sensitive dormant seeds that germinate in response to red light (R), and 3) light-insensitive dormant seeds that fail to germinate after 5 days of continuous R. Seeds of all three germination types were found on individual plants of both species. Less than 5% were nondormant, 50 to 60% were light-sensitive primary dormant, and 35 to 45% were light-insensitive primary dormant for diffuse and spotted knapweed seeds. Seeds collected from different sites, and from individual plants within sites, had different germination levels in darkness and following exposure to two minutes of R. Exposure to R stimulated germination in each case.

The mean levels of primary dormancy over all diffuse and spotted knapweed sites were 78 and 84%, respectively. The germination behavior of seeds collected from individual plants was similar to samples collected from different sites for both species. Freshly harvested seeds from individual spotted knapweed plants generally possessed higher levels of primary dormancy ($\overline{X}$ = 93%) than those from individual diffuse knapweed plants ($\overline{X}$ = 71%).

Phytochrome is a cytoplasmic protein with a covalently attached linear tetrapyrole chromophore that regulates flowering and seed germination. The phytochrome molecule exists in two different conformations depending on the wavelength of light of the last exposure. The R absorbing form, P_r, has maximum absorbance in the 600-680 nm range and is the inactive form. Upon exposure to R phytochrome undergoes a conformational change to the active or P_{fr} form which absorbs light in the far-red (FR) region with maximum absorbance in the 720-740 nm range. Phytochrome in the P_{fr} form undergoes dark reversion to the inactive P_r form (Song, 1984).

The demonstration of R/FR reversibility implicates involvement of the phytochrome system in light-sensitive seed germination (Smith, 1982). Nolan and Upadhyaya (1988) showed that light-sensitive dormant diffuse and spotted knapweed seed germinated in response to R and that exposure to FR negated this effect. Exposure to FR did not establish a light requirement in imbibed seeds that were initially non-dormant, as germination following FR was not significantly different from dark controls.

The level of germination following any sequence of light exposures for light-sensitive knapweed seeds depends on the light quality of the last exposure. Exposure to R for just 2 minutes was adequate to stimulate the germination of all dormant seeds from some individual plants, however longer exposures were required for maximum germination of seeds collected from other individual plants. When the duration of R exposure was increased from 2 minutes to 1 day, germination increased from 15 to 53% for diffuse knapweed, and from 15 to 63% for spotted knapweed. Further increases in R duration from 1 to 5 days did not increase germination.

Watson and Renney (1974) reported no difference in germination of diffuse and spotted knapweed seed when exposed to alternating light and dark versus dark incubation. In fact continuous light significantly reduced the germination of both species. Nolan and Upadhyaya explained this discrepancy by noting that they observed a loss of the light requirement in several samples of seeds used in preliminary studies following several months storage at room temperature. An increase in germination following dry after-ripening was reported by Watson and Renney (1974). Storage of seeds at -20° C arrested after-ripening in the seeds used by Nolan and Upadhyaya (1988) and maintained a high level of primary dormancy. Alternatively, chance collection from plants bearing mostly nondormant seeds may explain the lack of light-sensitivity of spotted and diffuse knapweed seed reported by Watson and Renney (1974).

The effect of gibberellic acid (GA_3) and excision on germination of diffuse and spotted knapweed seed was also reported (Nolan and Upadhyaya, 1988). Germination in darkness increased with increasing GA_3 concentration. There was no synergism between light and GA_3 on knapweed seed germination. Germination of diffuse and spotted knapweed seed was 100% following excision of the distal end of the seed.

Polymorphic germination behavior is a common phenomenon among weed species which insures seed germination for an extended period of time (Bewley and Black, 1982). Germination polymorphism occurs in both diffuse and spotted knapweed which exhibit three distinct types of germination behavior. Knapweed seeds germinate in the spring and fall (Watson and Renney, 1974). Nondormant and light-sensitive seeds that don't become buried or shaded by other plants would likely germinate immediately following dispersal in the fall if temperature and moisture levels were adequate. Delay of germination until the spring would be facilitated by dormancy, retention of seeds in the capitula over the winter, or seed dispersal late in the season when low temperatures prevent germination. Seed dormancy would further distribute germination over time by allowing seeds to become incorporated into a persistent seed bank in the soil (Fenner, 1985).

Differences in amounts of non-dormant seeds (seeds germinating in the dark) among individual plants within sites could be due to genetic variability between plants or differences in environmental conditions experienced by the maternal plant during seed development. The asynchronous flowering behavior of the knapweeds increases the possibility that variability in environmental conditions prior to seed dispersal leads to germination polymorphism. Variability in the rate or duration of the after-ripening period following seed maturation is one possible explanation for differences in dark germination (Fenner, 1985).

The higher average levels of primary dormancy in spotted knapweed compared to diffuse knapweed might be the result of the inherent differences in seed dispersal in the two species. Diffuse knapweed seeds are retained on the plant for prolonged periods because the capitula, except for a small distal opening, remain closed (Watson and Renney, 1974). Conversely, spotted knapweed seed heads open widely when mature and the seeds are loosely held in the capitula so they are dispersed quickly. Consequently, spotted knapweed seeds would undergo less after-ripening in the capitulum than seeds of diffuse knapweed. If seed germination behavior of the two species is comparable at the time of maturation, and primary dormancy is lost during after-ripening, the capability of diffuse knapweed to retain seeds in the capitula for longer periods would favor after-ripening resulting in lower levels of primary dormancy in field populations of this species.

Seed germination characteristics may influence the geographic range of adaptation of a species (Bewley and Black, 1982). Knapweed infestations are generally found in areas experiencing soil disturbance, overgrazing, or seasonal drought (Harris and Cranson, 1979; Popova, 1960; Watson and Renney, 1974), and are uncommon in irrigated, shaded, or moist areas (Meyers and Berube, 1983; Watson and Renney, 1974). All of the above factors affect the abundance of vegetative cover, which in turn influences the quality of light incident upon seeds present on the underlying soil surface. The absorption of the red wavelengths of energy in sunlight by chlorophyll in a plant canopy causes a decrease in the R:FR (660:730 nm) ratio as the leaf index increases (Frankland, 1981). Rangelands dominated by bunchgrasses provide favorable conditions for knapweed seed germination due to bare areas between plants that allows R to reach the soil surface.

The effects of stratification, temperature, and water stress on spotted knapweed seed germination were investigated by Eddleman and Romo (1986). Optimum temperature range for germination was 15 to 25° C.

Temperatures above or below this range resulted in reduced germination suggesting an adaptation that minimizes germination in late summer or early fall when seedling mortality may be high under conditions of transient moisture. Low temperature-induced dormancy may be an adaptation that restricts germination in late fall, when slow growth rates could lead to seedling mortality. High mortality of spotted knapweed seedlings has been noted during severe winters (Watson and Renney, 1974) when extreme low temperatures and low snow accumulation occur simultaneously. Widespread winterkill of spotted knapweed was noted for the winter of 1988-89 in western Montana (personal observation). However, the observed winterkill reduced plant density only slightly.

Immediately after harvest, stratification for 30 or 60 days alleviated a portion of the dormancy in spotted knapweed seeds incubated in the dark (Eddleman and Romo, 1988). Seeds of spotted knapweed under field conditions are subjected to cold-dry and cold-wet conditions during the fall and winter. Stratification increased spotted knapweed seed germination at both supraoptimal and suboptimal temperatures (Eddleman and Romo, 1988). Loss of dormancy at colder temperatures would enhance early emergence of seedlings in the spring. This would maximize the period of available soil moisture for newly developing plants and avoid competition from later emerging species. Such adaptations such as this that place seedlings in a favorable time-moisture relationship are critical for establishment of seedlings on grassland sites (Harris, 1967). Dry after-ripening and stratification increase seed germination of diffuse and spotted knapweed seed.

Plant Development

Spotted and diffuse knapweed bolt in May after overwintering as rosettes. Diffuse knapweed produces a single branched stem, two year old spotted knapweed plants produce one to six stems per plant, and older plants typically produce more than a dozen branches (Watson and Renney, 1974). Rosettes begin to bolt and immature flowers are first observed in mid-June. Stems and branches elongate and flower heads continue to appear on the end of each branch throughout the summer. Flowering begins in mid-July, about 2 weeks earlier for spotted knapweed than for diffuse knapweed. Individual flowers remain open for 2 to 6 days. The *Centaurea* species are cross-pollinated by insects and mature seeds are produced 18 to 26 days after fertilization (Watson and Renney, 1974).

Diffuse and spotted knapweed are adapted to a wide range of soil types. Susceptibility to invasion is directly related to the degree of disturbance of the soil surface. However, these two species are not adapted to cultivated land or land under irrigation. Spotted and diffuse knapweed thrive in areas where an arid period occurs during the summer months (Harris and Cranston, 1979). Open habitats are preferred, although spotted knapweed will invade disturbed forest soils at relatively high altitudes.

Spotted knapweed reproduces vegetatively and is classified as a short-lived perennial (Watson and Renney, 1974). Lateral shoots emerge 2.5 to 7.5 cm from the mother plant and form rosettes. Plants normally survive for 3 to 5 years which accounts in part for the dense stands formed by spotted knapweed. Diffuse knapweed is classified as a biennial or sometimes as a triannual (Watson and Renney, 1974).

Boggs and Story (1987) studied the population age structure of spotted knapweed in Montana. Life span and mean age of spotted knapweed plants were determined by counting annual rings. Regardless of environmental conditions of the season, one ring of secondary xylem was added to the taproot of known-age spotted knapweed plants annually. Root ring counts at six study sites indicate spotted knapweed plants can live for at least 9 years. The average age of spotted knapweed plants, excluding plants less than 1 year old, ranged from 3.4 years in 1984 to 5.0 years in 1985. Based upon these data and the definition of a perennial as any plant living longer than 2 years (Hitchcock and Cronquist, 1978), Boggs and Story suggest that spotted knapweed should be classified as a perennial rather than either a biennial or short-lived perennial.

Seed Production and Dissemination

Massive seed production is a major competitive device of the knapweeds. Watson and Renney (1974) reported that diffuse knapweed produced 925 seeds per plant when grown on rangeland and 18,248 seeds when grown under irrigation. Spotted knapweed produced 436 and 25,263 seeds per plant when grown under dryland and irrigated conditions, respectively.

Schirman (1981) reported that diffuse knapweed is adapted to drier climates than spotted knapweed. In a 3-year study, which included prolonged drought conditions, diffuse knapweed seed production was more stable than spotted knapweed. Diffuse and spotted knapweed produced approximately 30,000 and 48,000 seeds/m^2, respectively. The higher seed production by spotted knapweed was due to multiple flower stem production. While there was no correlation between spotted knapweed height and the number of flower shoots or flower heads produced per plant, there was a correlation found between the number of flower heads and shoot production per plant (Story, 1976).

Spotted knapweed plants produced 32 seeds per head and 29 heads per plant (approximately 1,000 seeds per plant) (Story, 1976). If 80% seed survival is assumed (Schirman, 1981; Watson and Renney, 1974), the soil reservoir of spotted knapweed seed increases exponentially each year. The longevity of spotted knapweed seed viability has not been determined. However, preliminary results by Schirman (1984), Chicoine (1984), and Lacey (1985) indicate that buried spotted knapweed seed remains viable in the soil for more than 5 years.

Each *Centaurea* species has a unique method of seed dispersal. Diffuse knapweed plants are commonly globe-shaped at maturity and have an abcission layer on the stem near the soil surface enabling them to break loose and tumble (Watson and Renney, 1974). The capitulum of diffuse knapweed is more constricted than spotted knapweed which favors seed retention prior to tumbling. Seeds are released as the plant rolls along the ground. Spotted knapweed does not disseminate its seeds over long distances since the flower stems do not tumble and the pappus is too small to permit wind dissemination of the seed. The bracts enclosing the flower heads begin to open approximately 3 weeks after maturity (Watson and Renney, 1974). As the relative humidity fluctuates the bracts open and close which loosens the seeds causing them to rise to the top of the capitulum (Watson and Renney, 1974). Seeds are disseminated by a flicking motion caused when plants are moved abruptly which scatters the seed for distances of up to 1 meter (Strang et al., 1979).

Spotted knapweed is moved from one locality to another by man and animals. The bristles of the pappus enable the seed to loosely adhere to animal hair or fur allowing transport over short distances (personal observation). Motorized vehicles are primarily responsible for the rapid long distance spread of spotted knapweed seed throughout North America (Mass, 1985; Watson and Renney, 1974).

Spotted knapweed seed floats on water due to its waxy pericarp and bristly pappus which tends to trap air (personal observation) and so waterways also serve as transportation avenues for seed.

Allelopathy

There is some evidence that *Centaurea* species utilize allelopathy to maintain stand densities. Fletcher and Renney (1963) partially characterized the suspected allelochemical as an indole. The highest concentration of allelochemical was found in the leaves. Russian knapweed (*Centaurea repens* L.) had the highest concentration of the allelochemical, followed by diffuse and spotted knapweed. Soils from Russian knapweed infestations retarded growth of tomato (*Lycopersicon esculentum*) and barley (*Hordeum vulgare* L.).

Kelsey and Locken (1987) used chloroform extraction to obtain compounds from spotted knapweed tissue. Column chromatography of extracts provided 17 fractions that were